NIST Conference on Microarray Standards

Validation of the Affymetrix Microarray System and the Challenges Faced

R D Hockett, MD
Senior Clinical Research Physician
Group Leader Genomic Medicine

Answers That Matter.

NIST Standards Conference

- Brief Description of Clinical Trial
- Validation plan for the Affymetrix Array
  - Defining Variability
  - Establishing Control Ranges
  - Testing controls

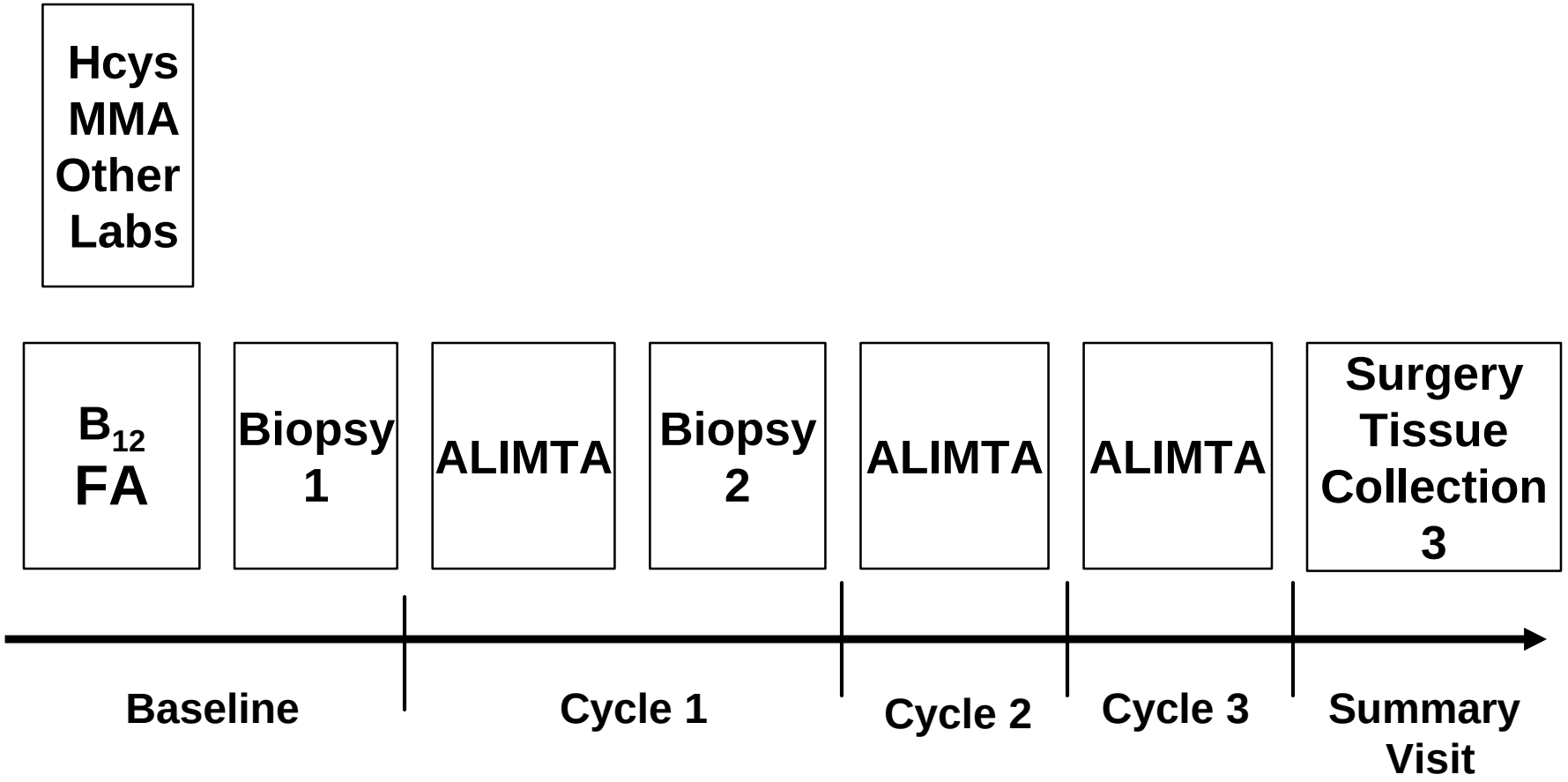
- Investigating Parameters around RNA Quality

Study Objectives

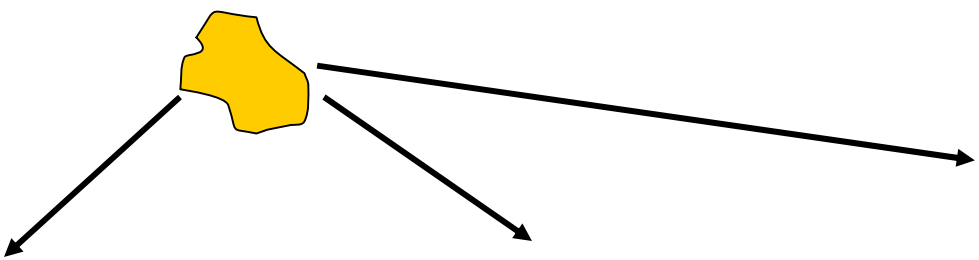
- **Correlation of tumor tissue derived biomarkers and clinical outcome at:**
  - **Baseline (prior to treatment)**
  - **24 hrs after first dose of ALIMTA**
  - **After 3 cycles of ALIMTA**

- **Clinical response and standard efficacy and safety end-points**

Study Design



Distribution of Tissues



Storage

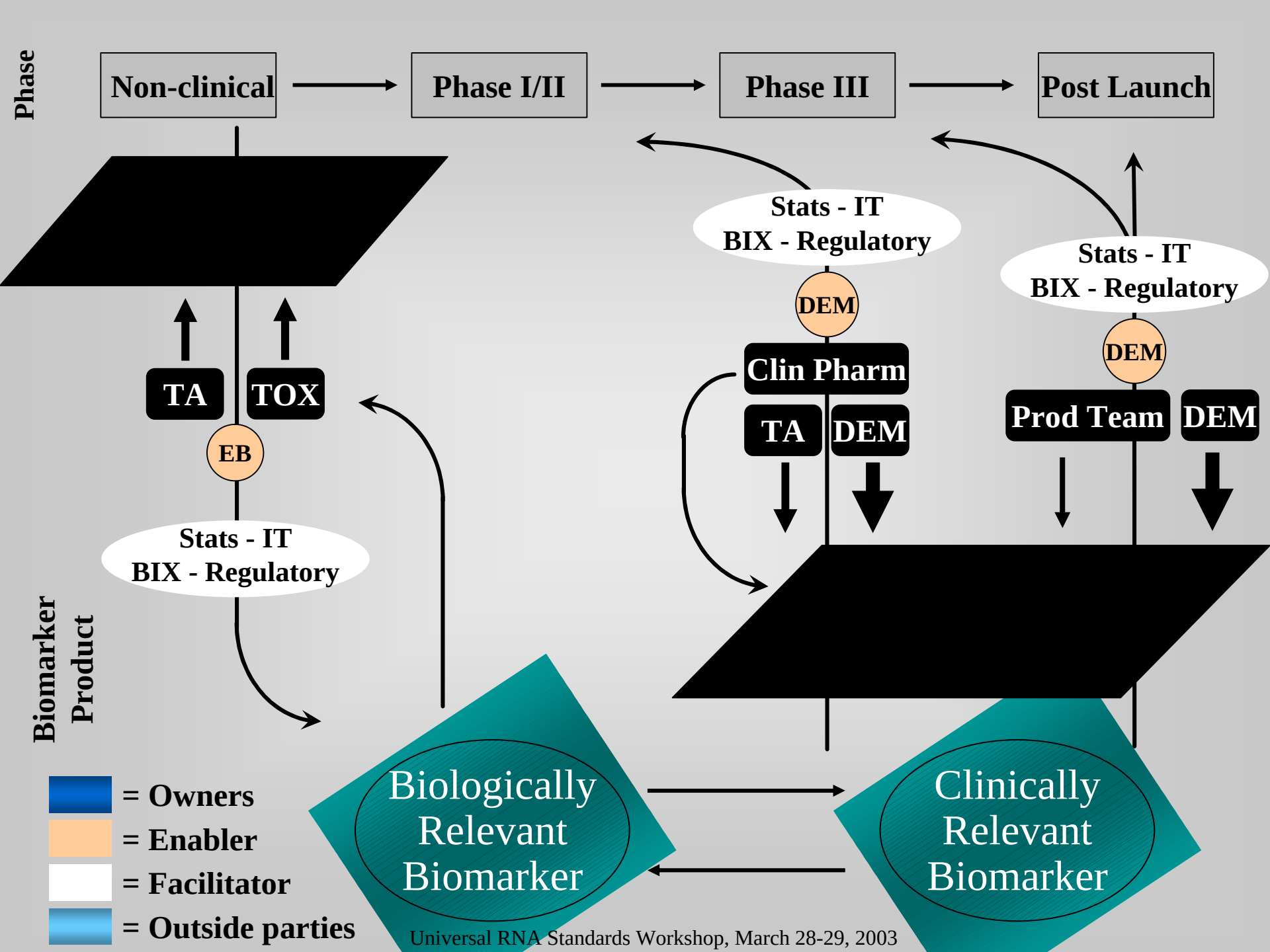
Array Profiling

Histology, Immunohistochemistry, and/or FISH

- H & E
- TS
- DHFR
- GARFT
- p53
- erbB-2

Quantitative PCR

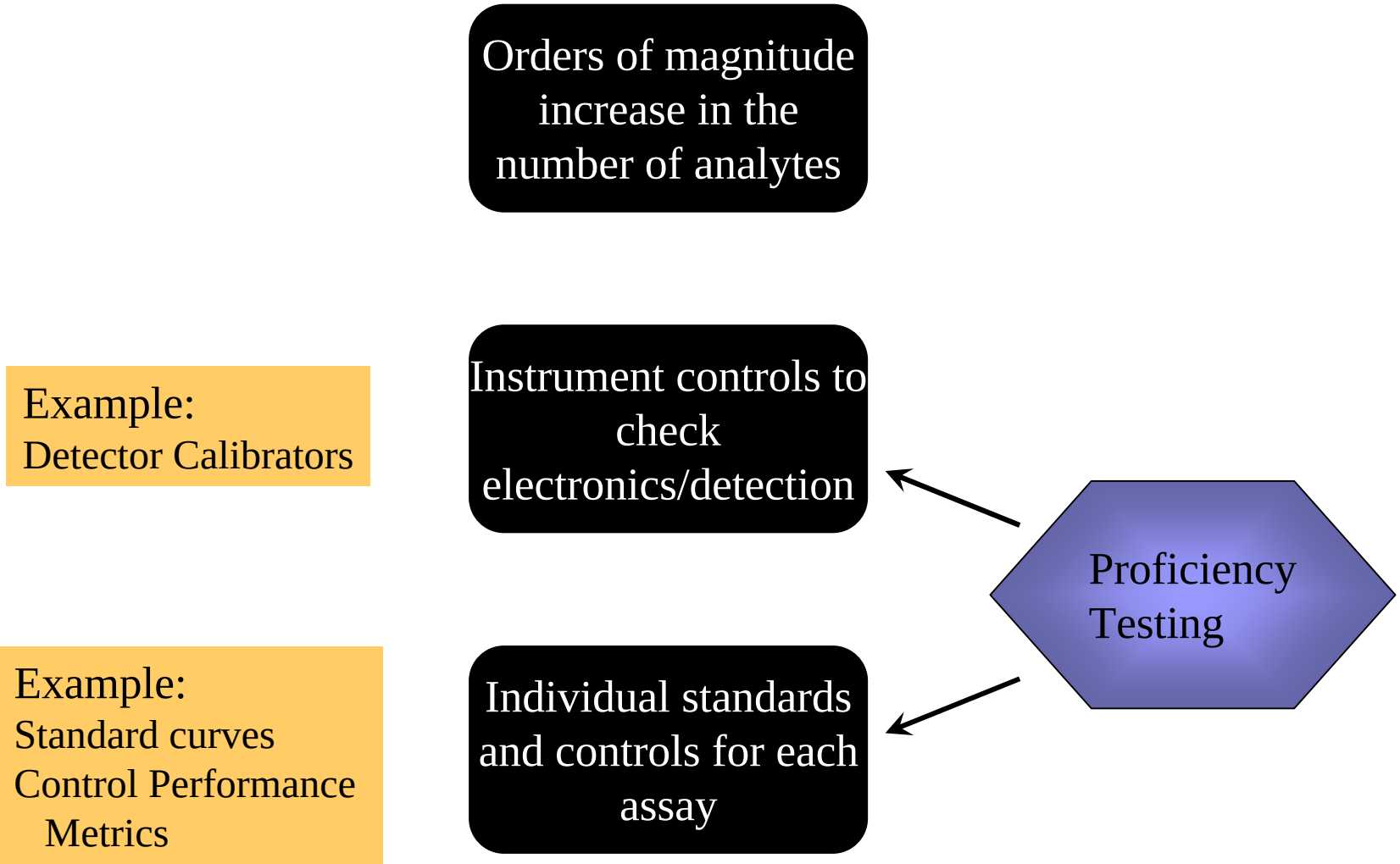
- TS
- DHFR
- GARFT
- p53
- erbB-2
- b-actin
- others


Phase
Non-clinical
Phase I/II
Phase III
Post Launch
TA
TOX
EB
Stats - IT
BIX - Regulatory
Stats - IT
BIX - Regulatory
DEM
Clin Pharm
TA
DEM
Stats - IT
BIX - Regulatory
DEM
Prod Team
DEM
Biomarker
Product
Biologically
Relevant
Biomarker
Clinically
Relevant
Biomarker
= Owners
= Enabler
= Facilitator
= Outside parties

Clinical Validation of Microarrays

- Validation Process
  - Establish Instrument Parameters
  - Comply with CFR 21 part 11
  - Define Variability
    - Accuracy
    - Precision
  - Set Standards
  - Establish Control Parameters
    - Acceptance/Rejection Criteria
  - Validation Document

Clinical Validation of Microarrays



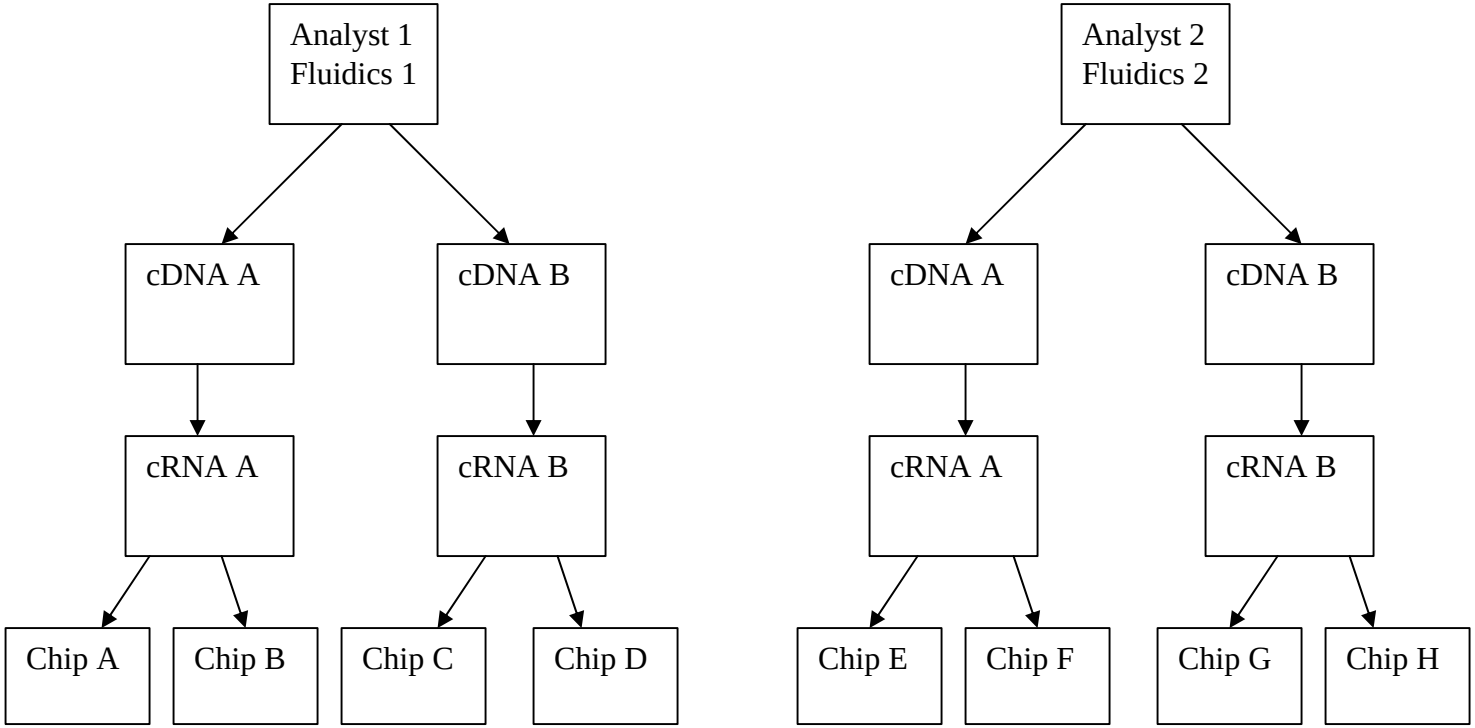
Affymetrix Microarray Validation

- Experiment #1 - Define procedure variability
  - How many times should each step be run for a valid result?
    - How many extractions?
    - cDNA syntheses?
    - cRNA labelings?
    - Chips?
  - Define variability of each step, including technologist and fluidics workstations

Affymetrix Microarray Validation

- 7 runs will be completed by each technologist
  - For each run the analysts will be working side by side on the same day.
  - Each analyst will use 4 chips
  - Two lots of chips will be used
    - 4 runs one lot - 3 runs second lot
- The fluidics stations will be alternated between analysts each run.
- The design for experiment 2 will be finalized using the results from experiment 1

Affymetrix Microarray Validation



56 Chips Total

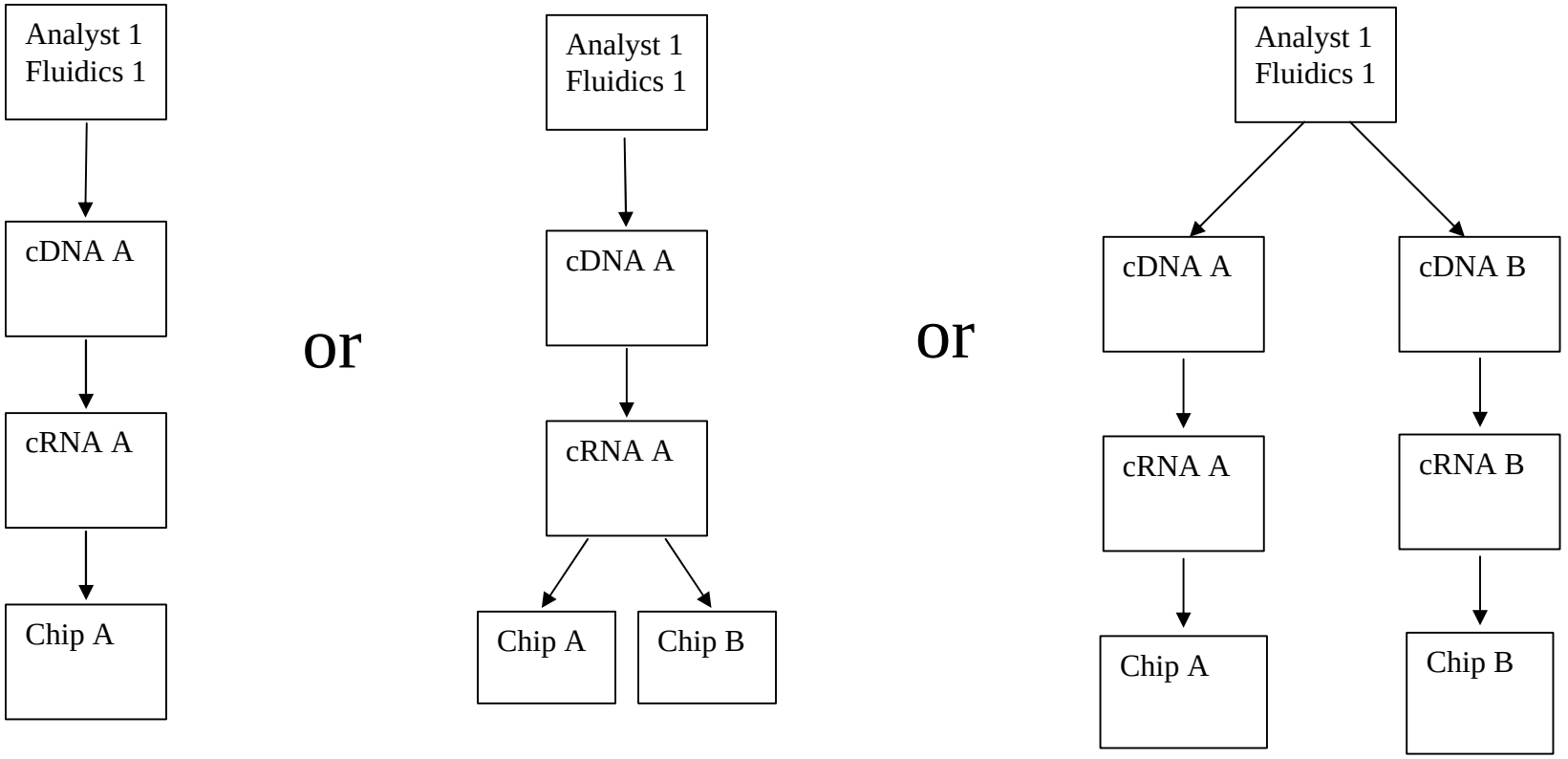
Data will determine subsequent procedure

24 Chips planned from tissue source

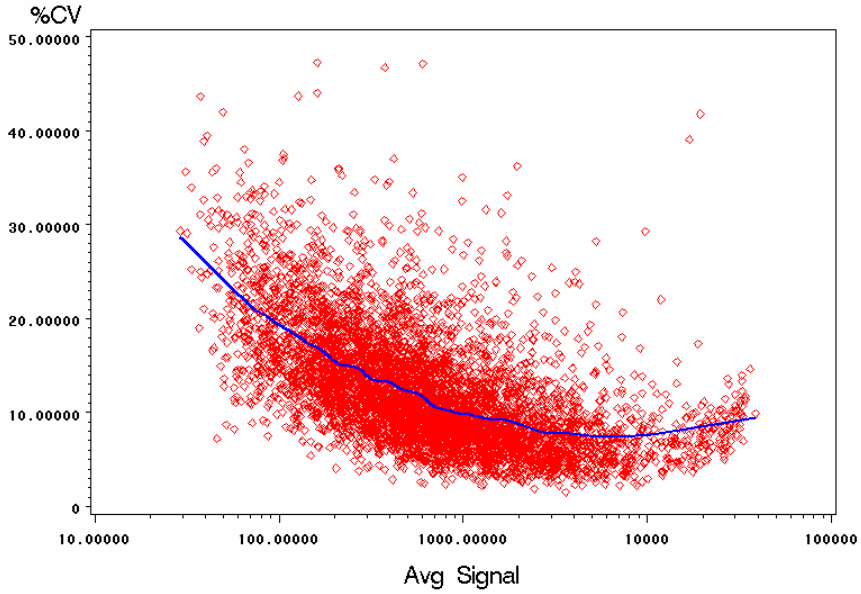
Affymetrix Microarray Validation

- Experiment #2 - Establish control parameters
  - During clinical use, one of every 8 chips will be control
  - All genes checked for acceptance/rejection criteria
    - 2/3 of genes must be within acceptable limits or entire run of 8 chips must be repeated
  - Limits set by running control 25 - 30 times over different days by different analysts
    - Procedure set by experiment #1

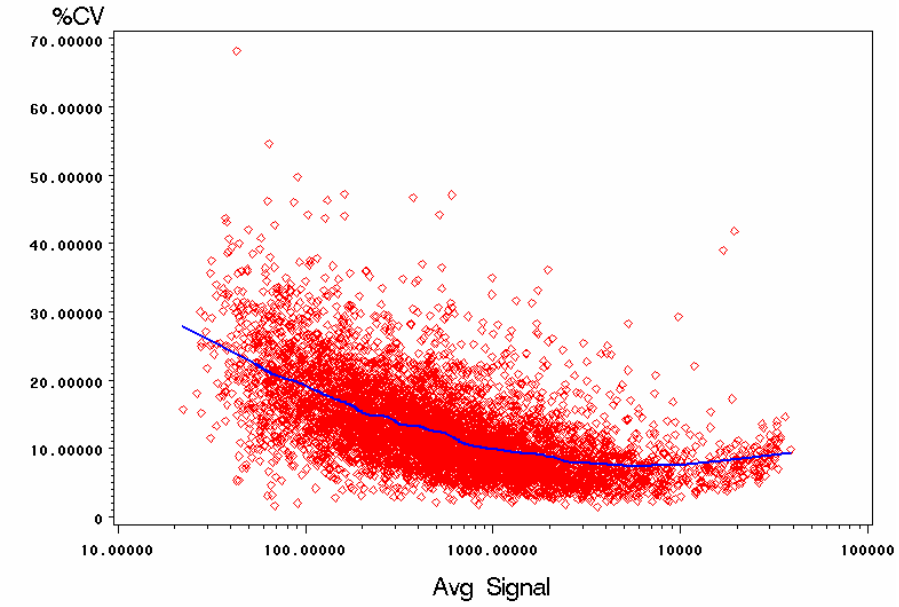
Affymetrix Microarray Validation



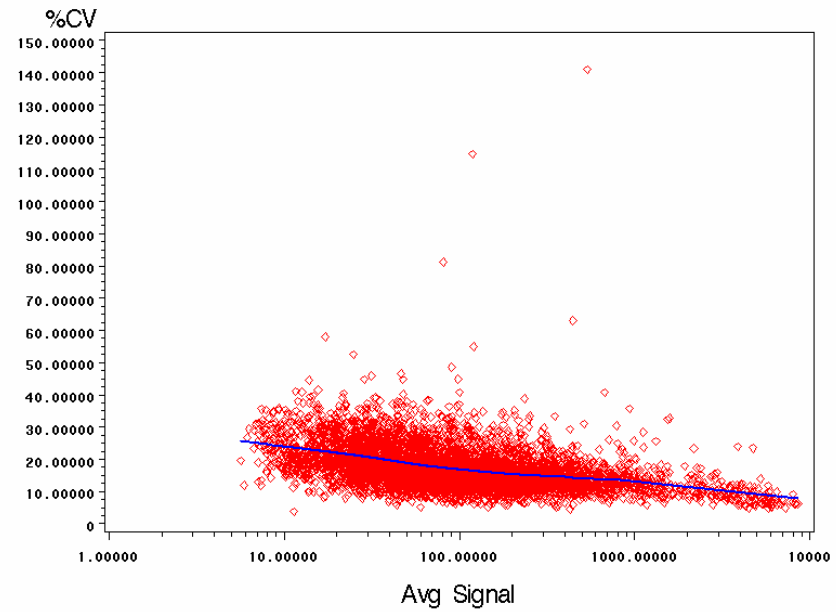
Run 1



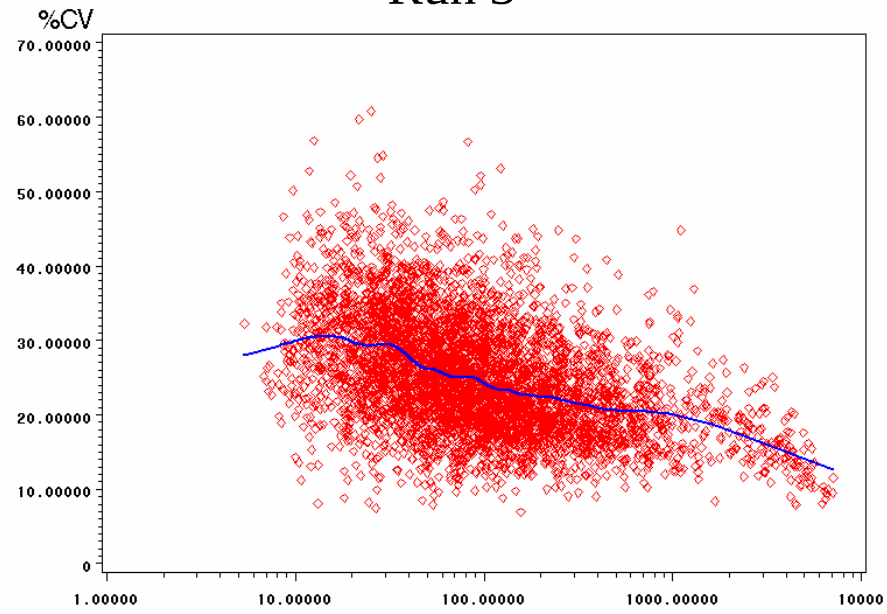
Run 1 with ≥ 2 P/M



Run 2

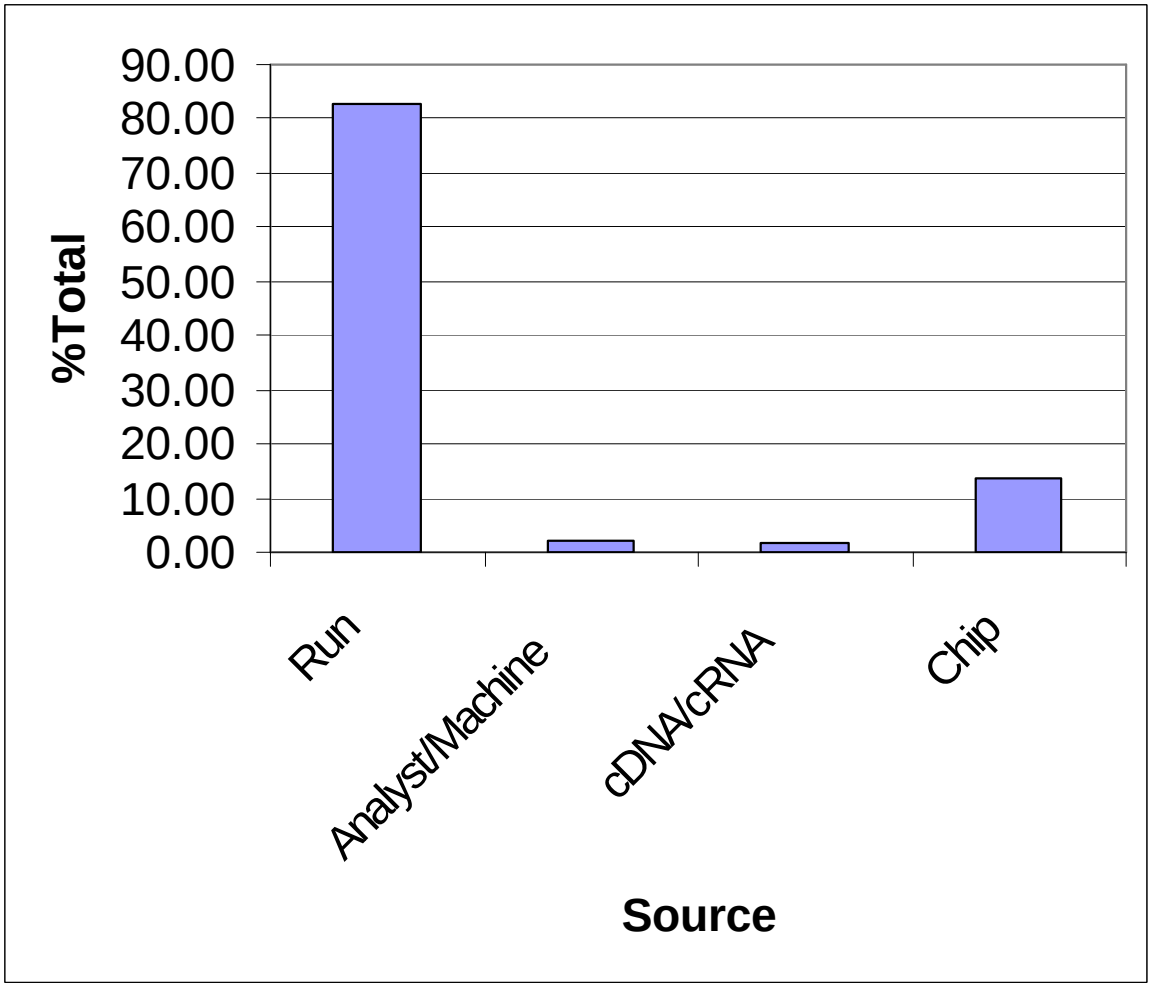


Run 3



Average %Variance Explained Across Genes

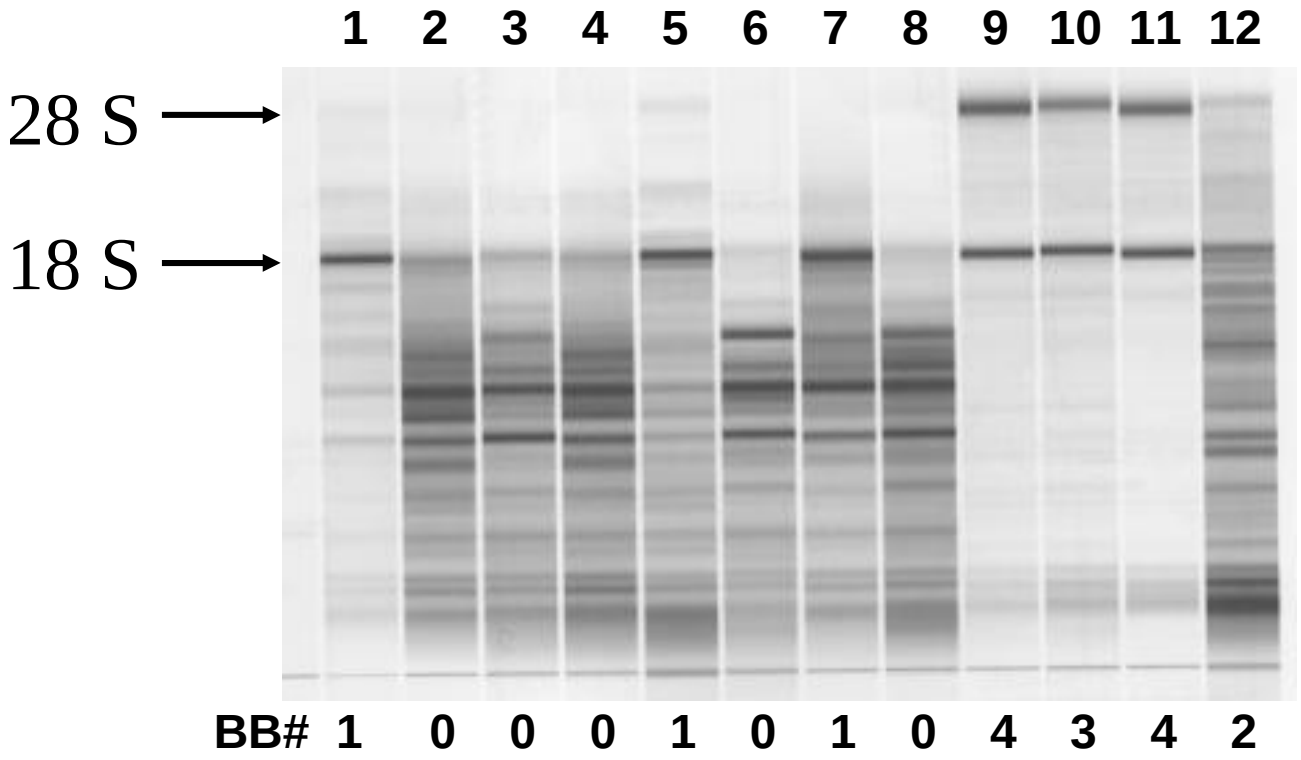
Effect	%Total Variance
Run	82.74
Analyst/Machine	1.96
cDNA/cRNA	1.80
Chip	13.50



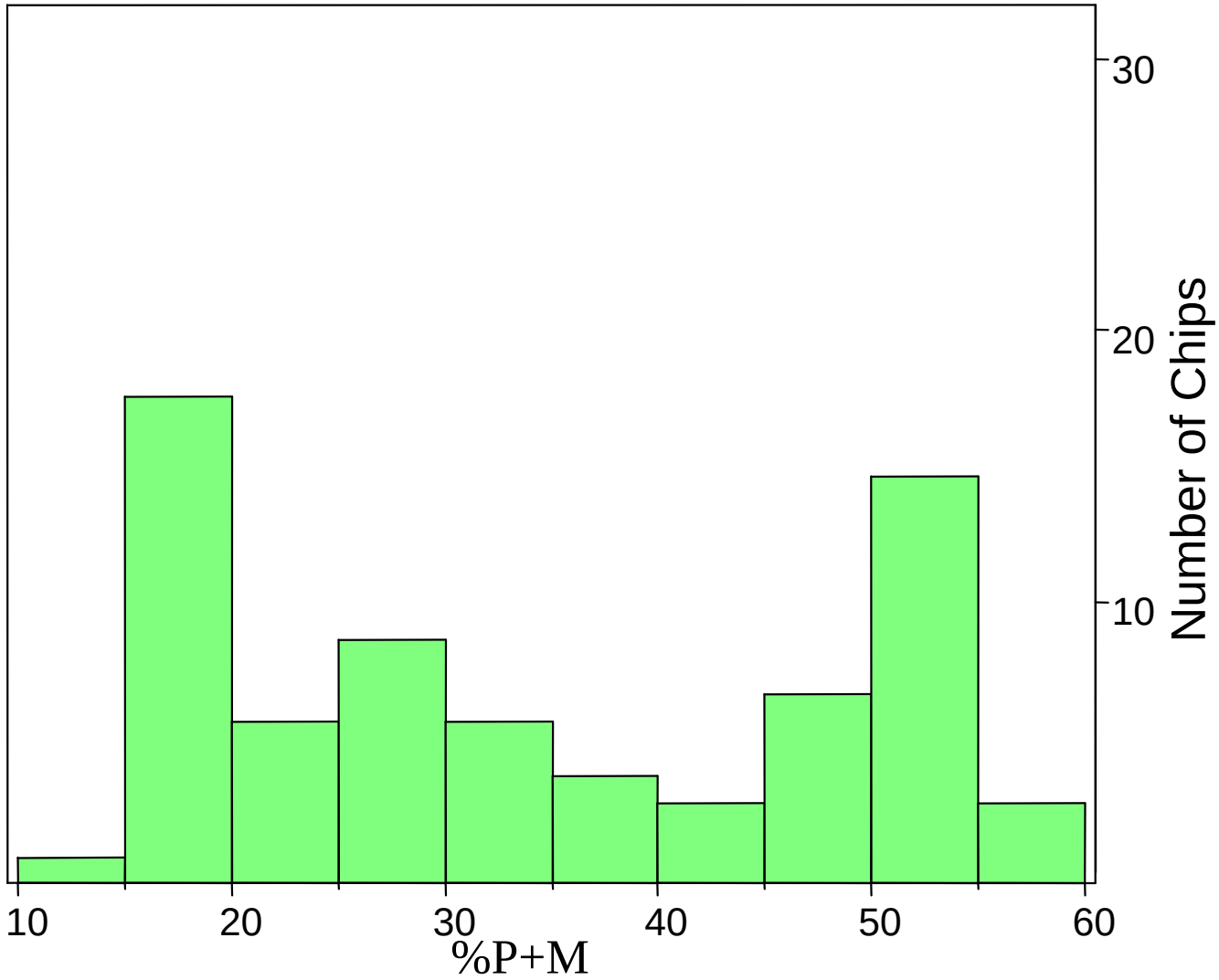
Sample Usefulness

- When collecting clinical samples, how much RNA degradation can be present and still get meaningful results?
  - Pristine?
  - Both 28S and 18S present?

Sample Quality



Sample Quality



Sample Quality

Group	Sample ID	%P+M*	BB#	Group	Sample ID	%P+M*	BB#
1	LES-101	56.24	4	3	LES-39	54.13	3
1	LES-123	53.78	2	3	LES-98	53.61	4
1	LES-105	53.35	1	3	LES-34	52.56	3
1	LES-106	51.89	3	3	LES-33	51.60	4
1	LES-100	49.50	3	NA	LES-46	51.57	4
1	LES-96	47.83	2	3	LES-63	45.46	1
1	LES-153	36.62	1	3	LES-103	45.28	1
1	LES-131	33.78	1	NA	LES-43	44.60	2
1	LES-3	32.74	2	2	LES-61	33.57	1
1	LES-57	29.11	1	3	LES-134	31.74	2
1	LES-45	29.09	0	3	LES-86	22.96	1
1	LES-50	26.52	1	3	L3S-5	21.08	1
1	LES-155	25.84	1	3	LES-7	19.87	0
1	LES-62	25.51	1	3	LES-92	19.02	1
1	LES-22	19.63	1	2	LES-64	18.82	1
1	LES-27	17.55	0	3	LES-90	17.89	1
1	LES-88	17.31	0	2	LES-6	17.74	0
1	LES-59	15.48	1	2	LES-4	14.94	1

*Average of two chips.

BB#	Proportion "Useful"	% Usable
0	0/5	0
1	4/18	22%
2	3/5	60%
3	4/4	100%
4	4/4	100%

r = 0.78

p = 1.90E-08